



Section 4:

Pathogen and Vector Monitoring and Action Thresholds

QUANTITATIVE ASPECTS OF THE TRANSMISSION OF *XYLELLA FASTIDIOSA* BY THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

Transmission of *Xylella fastidiosa* (*Xf*) by the glassy-winged sharpshooters (GWSS) involves a series of events from acquisition of the bacterium to inoculation of *Xf* to a new host. While this process is often over-simplified, certain insect/pathogen interactions may be necessary to achieve a successful transmission event and the number of *Xf* cells acquired or inoculated may govern whether or not transmission will occur. In our preliminary studies, neither higher titers of *Xf* nor longer feeding periods by GWSS result in higher rates of transmission nor a greater number of bacteria transmitted.

INTRODUCTION

Solutions to Pierce's disease (PD) are coming out of an understanding of basic biological aspects of the vector, the pathogen, their hosts, and especially the interactions among these three divergent organisms that culminate in a disease epidemic. The most important of these interactions is the transmission of the pathogen by the vector to a non-infected plant. Transmission is a product of vector acquisition of the pathogen from an infected plant, and inoculation of the pathogen into a non-infected plant. It is a complex process involving sharpshooter host finding and feeding behaviors, and probabilities that a critical titer of bacterium will be acquired from an infected host by a feeding sharpshooter, and once acquired, will be inoculated into an uninfected host. In addition, for an inoculation event to lead to infection, a critical titer of bacterium must be inoculated into plant tissue that supports reproduction and movement.

Recent advancements in technology allow us to examine quantitative aspects of *Xf* transmission with high sensitivity, unlike traditional means. This includes two techniques we have mastered in our laboratories. First, we are currently using a quantitative real-time (QRT PCR) technique in conjunction with commercially available DNA extraction kits to detect and quantify low titers (currently ca 5×10^1 cells) of *Xf* in plant and insect tissue [2]. Second we have developed a low-cost method to rapidly extract DNA from GWSS and plant tissue in 96-well micro-titer plates.

Species of sharpshooters differ widely in their transmission efficiency, which ranges from a high of over 90% for the blue-green sharpshooter (*Graphocephala atropunctata*) to 1% for several others including *Oncometopia facialis*, *Acrogonia virescens*, and *Homalodisca ignorata* [3]. Recently, rates of *Xf* transmission efficiency for the GWSS from grapevine to grapevine were found to be as high as 20% [1]. These observations bring up two questions: First, what aspects of *Xf* transmission by sharpshooter vectors vary in ways that cause a wide range in efficiencies among vectors? Second, can we exploit an understanding of transmission efficiency to reduce PD spread? We seek to understand quantitative aspects of *Xf* transmission by GWSS. We are hopeful that this unique approach to investigating the transmission of an insect-vectorized plant pathogen will lead to new tactics to manage disease spread.

OBJECTIVES

Our long-term goal is to understand quantitative aspects of the process of *Xylella fastidiosa* (*Xf*) transmission by *Homalodisca coagulata* (glassy-winged sharpshooter, GWSS) in order to develop a means of reducing the efficiency with which the pathogen is spread from an infected plant to a non-infected one. Our specific objectives for this project are to:

1. Determine relationship between the time a GWSS spends on a PD-infected grapevine and titer of *Xf* they acquire.
2. Determine the relationship between the time a GWSS spends in post-acquisition on a non-*Xf* host and titer of *Xf* they contain.
3. Determine the relationship between the time an infectious GWSS (ie, one that had acquired *Xf*) spends on a non-infected grapevine and the titer of *Xf* it inoculates into the grapevine.
4. Determine the relationship between the titer of *Xf* inoculated into a plant and the probability that it will become diseased.

RESULTS

Our preliminary laboratory experiments show that we can quantify the titer of *Xf* delivered to a stem by a single infectious GWSS immediately after a 24hr inoculation access period (IAP). In this experiment, field-collected GWSS adults were allowed to acquire *Xf* from grapevines showing Pierce's disease symptoms for a 72 hr acquisition access period (AAP). GWSS were then allowed access to cut chrysanthemum stems for 2, 4, 6, or 8 h. During this IAP, time lapse video was used to determine the amount of time GWSS feed on the stem and number of times the insect left the stem (indicating multiple

probing activities). In preliminary experiments, longer feeding durations did not influence the number of cells transmitted. Other data are too preliminary to present at this time.

CONCLUSIONS

We have the tools in place to determine transmission rates at the molecular level. Experiments are underway to determine the number of *Xf* cells that are transmitted under certain conditions. Until recently the molecular tools were not available to monitor the movement of single cells in the manner that QRT PCR allows. Almeida et al. [1] encountered difficulty in detecting levels of *Xf* in GWSS that can successfully inoculate a grapevine. That is, they found GWSS that were able to inoculate plants with *Xf* that did not test positive for the pathogen. The most reasonable explanation for these “false negatives” is that these GWSS harbored a titer of *Xf* that can cause infection in grapevines, but were below detection limits. Theoretically, one cell can cause a chronic infection; however, the probability is very low. We suspect the number of cells that are likely introduced into plants is greater than a single cell, but lower than the detection threshold of the method used by Almeida et al. [1], which is 10^2 cells. We need to embrace the molecular tools that are available to accomplish our objective.

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DEVELOPING A METHOD TO DETECT *XYLELLA FASTIDIOSA* IN THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

A rapid and reproducible technique to detect *Xylella fastidiosa* (*Xf*) in the glassy-winged sharpshooter (GWSS) is important for epidemiological studies, and monitoring programs in support of Pierce's disease management. Such a technique must be amenable to large samples sizes, while remaining sensitive enough to detect pathogen DNA in low amounts. In this study we have improved the speed of tissue extraction by developing a simple vacuum step that replaces labor and time-intensive tissue maceration, and is compatible with manufactured DNA extraction kits and a SYBR Green® based real-time (QRT) PCR system. No statistical differences in the ability to detect *Xf* were found among samples that were extracted using traditional maceration vs. our vacuum extraction method. Further experiments using our vacuum extraction methods detected no significant differences among samples immediately extracted, or stored for 10 d at -4°C, dry or in mineral oil. In another experiment we placed *Xf*-fed GWSS on yellow sticky cards in a sunny location for 0 to 6 d. We found that there was no significant reduction in our detection capabilities for insects left on the cards.

INTRODUCTION

Grapevines infected with *Xylella fastidiosa* (*Xf*), the bacterium that induces Pierce's disease of grapevine [12], usually die within three to five years after infection due to the occlusion of xylem vessels [17]. The glassy-winged sharpshooter (GWSS) has recently become an important vector of *Xf* in California, spreading *Xf* to grapevines that traditionally had little or no Pierce's disease [2, 17]. This vector can disperse widely [5], and has a large host range [18] resulting in alarming spread of *Xf* to new areas [11]. The presence of GWSS in new regions of California, greater incidences of *Xf*-induced diseases in several crops, including grapevine [15], almond [1], oleander [10], and the threat of citrus variegated chlorosis (not currently found in the US) has lead to great concern over the ecology of this pest/pathogen interaction.

Over the past several years control programs have focused on reducing pathogen spread by managing vector populations [18]. Improvements of these strategies can be achieved through studies examining patterns of disease epidemiology [15, 20], and GWSS population densities and dispersion [5, 11, 21]. Most epidemiological studies of this system have involved *Xf*'s interaction with host plants [3, 6, 15, 20] or the population and behavioral ecology of the pest insect [5, 11]. Investigations of the interactions between *Xf* and insect vectors have largely been limited to laboratory and greenhouse studies [2, 4, 10].

Molecular protocols, such as PCR, to detect *Xf* in plants have been developed and are currently being used in epidemiological studies in other disease systems [8, 9, 14, 16, 19, 20]. Unfortunately, methods adapted to detect *Xf* in insects are inefficient. Detection methods designed for epidemiological studies, from collection of insect specimen to analysis of samples for the presence of *Xf*, need to be rapid, reproducible, inexpensive, and amenable to large sample sizes. We recently developed a DNA extraction protocol using the DNeasy tissue extraction kit (Qiagen Inc.) in conjunction with a SYBR Green® based real-time (QRT) PCR system to detect *Xf* in infectious GWSS [4]. Using this protocol, we reliably detected 50-500 *Xf* cells with GWSS background. This method used labor-intensive maceration of tissue to extract *Xf* from insect tissue where the bacterium resides in infectious insects [7]. The speed and efficiency of this method could be improved by simplifying this extraction step.

OBJECTIVES

Our overall goal is to develop a method of detecting *Xf* in infectious GWSS that would allow us to conduct epidemiological studies and optimize plant protection. To this end, the objectives for this study are to develop an efficient method to remove *Xf* cells from the foregut and mouthparts of GWSS for PCR based detection.

RESULTS

In this study we tested a vacuum extraction protocol for removal of *Xf* cells from GWSS foreguts for detection by QRT PCR. GWSS adults, collected from orange trees at the University of California, Riverside, were placed in rearing cages and allowed to feed for a 6 d acquisition access period on cuttings of *Xf*-infected grapevines that showed Pierce's disease symptoms. GWSS heads were removed, and because they float, an insect pin was placed through the back of the insect head and forced through the frons, so that the tip of the pin protruded slightly. The pinned head was then placed in a microcentrifuge tube (one per tube) and 500µl phosphate buffered saline (PBS) was added to the tube so that the head was completely submerged. Tubes were loaded into a tube rack and placed in a glass vacuum desiccator. With the desiccator lid in place, vacuum was applied to 20 bars slowly, to keep buffer from being displaced from its tube, and held for 15 s. Then,

the slow release valve was opened and pressure was slowly returned to ambient. The vacuum application and release was repeated 3 times. In this way, the insect's foregut and mouthparts were flushed out with PBS. The pinned heads were removed and DNA was extracted from the fluid using the DNeasy Tissue kit (Qiagen Inc.). QRT PCR was conducted as described earlier.

To compare our vacuum extraction method to a more conventional maceration technique, heads from GWSS infected with *Xf*, as above, were either macerated in PBS buffer with a pellet pestle in a disposable 1.5mL microcentrifuge tube (Kontes Glass Company, Vineland, NJ) or vacuum extracted in PBS buffer. In further experiments insects were collected and immediately extracted (n=24) as previously described or stored at -4°C for 10 d either submerged in mineral oil (n=24) or not (n=24). Finally, infectious GWSS were placed by hand on yellow sticky cards (Trécé Inc., Adair, OK). Yellow sticky cards were placed outside in a sunny location. GWSS were removed from the traps for DNA extraction at 0, 3, and 6 d after placement. DNA was extracted individually from GWSS heads using the vacuum technique and QRT-PCR was used for detection of *Xf*.

DNA Extraction

The vacuum extraction technique developed in this study improved the speed and efficiency of extraction. Extraction of DNA using traditional maceration with the Qiagen DNeasy tissue kit averaged 90 minutes for 24 samples. About 30-40 minutes of the extraction was preparing for and executing the maceration step of the procedure. Using the vacuum extraction technique we prepared 24 samples in an average of 15 min. The vacuum extraction technique neither improved nor compromised our ability to detect *Xf* in GWSS heads. No statistical differences were revealed between maceration-extracted and vacuum-extracted samples in any trial for either the number of positive samples or the relative amounts of *Xf* DNA measured (Table 1). However, in 5 of 6 trials mean positives and mean relative fluorescence levels were greater for macerated samples than vacuum-extracted samples (Table 1).

Table 1. Proportion of GWSS positive for *Xf*, and mean relative fluorescence using vacuum (VE) and maceration (MP) sample collection prior to DNA extraction (n=24).

Trial	Mean Positive ^a		Mean relative fluorescence ^b	
	VE	MP	VE	MP
1	0.458a	0.542a	1.137a	6.299a
2	0.464a	0.789a	1.728a	5.879a
3	1.000a	0.917a	0.112a	0.125a
4	0.917a	0.958a	0.001a	0.003a
5	0.750a	0.917a	0.009a	<0.001a
6	0.917a	0.792a	<0.001a	<0.001a

^aMeans in the same row followed by the same letter were not statistically different ($\chi^2 > 6.6$, df=1, $p > 0.359$).

^bRelative fluorescence correlates to cell number. Means in the same row followed by the same letter were not statistically different ($\chi^2 < 3$, df=1, $p < 0.01$).

Comparison of Sample Storage Methods

On either collection date, there were no significant differences in mean number of GWSS testing positive for the presence of *Xf* that could be attributed to the method of storage following GWSS collection (trial 1 $\chi^2 = 1.626$, df=2, $p = 0.443$; trial 2 $\chi^2 = 2.4$, df=2, $p = 0.3$) (Table 2).

Table 2. Comparison of *Xf* detection in GWSS following storage by three methods (n=24)

Trial	Storage method (n=24) ^a		
	Directly off Plant	-4°C (10 d)	-4°C in mineral oil (10 d)
1	0.875a	0.792a	0.917a
2	0.833a	0.750a	0.917a

^aMeans in the same row followed by the same letter were not statistically different (trial 1 $\chi^2 = 1.626$, df=2, $p = 0.443$; trial 2 $\chi^2 = 2.4$, df=2, $p = 0.3$).

Detection Capabilities Following Insect Trapping

Exposure to the elements after capture on sticky cards had little effect on the ability to detect *Xf* in GWSS samples (Table 3). Chi-square test for goodness of fit revealed no statistical differences among means from trial 1 (data taken 0, 3, and 6 days following capture, $\chi^2 = 3.069$, df=2, $p = 0.216$), or trial 2 (data taken 0, 3, and 6 days following capture, $\chi^2 = 2.845$, df=2, $p = 0.241$).

Table 3. Proportion of GWSS positive for *Xf* after outdoor exposure on a yellow sticky card.

Trial	Mean proportion of GWSS positive for <i>Xf</i> ^a		
	Day 0	Day 3	Day 6
1(n=49)	0.388a	0.429a	0.265a
2(n=30)	0.533a	0.333a	0.367a

^aMeans in the same row followed by the same letter were not statistically different (trial 1 $\chi^2=3.069$, df=2, $p=0.216$, trial 2 $\chi^2=2.845$, df=2, $p=0.241$)

CONCLUSIONS

Our study was conducted to find a means of accelerating a series of steps required to conduct epidemiological studies involving GWSS spread of *Xf*, while maintaining a high degree of detection sensitivity. Epidemiological studies require the examination of a large numbers of samples; therefore, an efficient testing protocol is necessary. Through our investigation, we improved the efficiency of *Xf* detection by streamlining DNA extraction and implementing a QRT-PCR-based detection system. The vacuum method was simple, requiring only that heads be removed, pinned into position, and covered with extraction buffer. While time efficiency is the most obvious advantage to using the vacuum extraction method, other advantages also exist which did not impact the studies reported here but may affect detection in field samples. First, no insect tissue is homogenized; it is likely that fewer PCR inhibitors are released to interfere with the PCR reaction and less non-template DNA would be extracted. These factors often hinder detection of pathogen DNA in low concentrations. Second, by flushing the content of the insect's foregut the search for the presence of *Xf* is being concentrated in the area of the insect that will most likely contain the organism of interest. QRT-PCR is a sensitive detection technique that allows low concentrations of bacteria to be detected in environmental samples [13]. Our QRT-PCR detection system improved detect an order of magnitude, from 500 *Xf* cells (with traditional PCR[4]) to 50 *Xf* cells per insect sample. The implementation of such a system is well suited for the detection of pathogen DNA in an insect vector.

A disadvantage of using a molecular technique like PCR for the detection of a pathogen in a host is that detection is based on the presence of pathogen DNA. Unfortunately this does not necessarily mean that the pathogen was alive at the time of collection; the presence of DNA confirms the presence of the pathogen in the host. While other techniques, such as culturing [2], determine the presence of live cells, the sensitivity of such a technique is lower than molecular techniques. The 5-10 d growth period required to see *Xf* colonies on a nutrient agar plate allows time for contaminants to overgrow the plate. Although specialized media are often used for growth, confirmation of bacterial identity is still needed. While morphological and colony growth characteristics are often used, genetically based identification is more reliable and discriminatory.

The mean number of GWSS testing positive varied between trials and between experiments. This was most likely due to natural variation in the ability of GWSS to harbor *Xf* which may be a function of both the insect's age and its exposure to other biotic and abiotic factor that influence the ability of the bacterium to colonize the foregut of GWSS. This does not compromise our objective which was to develop a detection protocol that could be used regardless of field conditions.

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MONITORING THE SEASONAL INCIDENCE OF *XYLELLA FASTIDIOSA* IN GLASSY-WINGED SHARPSHOOTER POPULATIONS

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ABSTRACT

The seasonal incidence of *Xylella fastidiosa* in GWSS populations will be examined using a combination of analytical and experimental techniques. Collections of live GWSS adults will be made at various locations in southern California throughout the year at regular intervals. Live insects will be confined individually to grapevine plants (var. Chardonnay) to determine what proportion from the field transmit *Xf*. Following a 3 day inoculation access period, each test insect will be processed accordingly for detection of *Xf* by PCR, ELISA, and/or culturing techniques. By examining sufficient numbers of insects from the field and comparing transmission test results to analytical results, the relative efficiencies of each technique at identifying infected or infectious insects will be determined. Moreover, the seasonal occurrence of infectious insects will be determined and may provide guidance for when to be most vigilant for protecting against primary spread of *Xf* into vineyards.

INTRODUCTION

The rate of *Xylella fastidiosa* Wells transmission in the natural environment is a fundamental component of the epidemiology of *Xf*, but one that is thus far poorly defined. As a xylem-limited bacterial pathogen of plants, *Xf* is dependent upon xylophagous leafhoppers for movement from one host to another. The rate that such movement occurs is determined by a large number of factors and interactions among plant hosts, vectors, and bacterial pathogen within the context of variable environmental conditions. Although the inherent complexity of vector-borne diseases defies whole-system approaches to epidemiological studies, specific parameters can be studied towards an overall understanding of vector-borne epidemiology. In the case of *Xf*, the number of leafhoppers feeding upon *Xf*-infected plants, the proportion of those that attain *Xf* through feeding, and the proportion of those that visit and ultimately inoculate uninfected host plants plays a critical role in the spatial and temporal dynamics of Pierce's Disease (PD) and other *Xf*-caused diseases. By investigating the proportion of glassy-winged sharpshooters (GWSS, *Homalodisca coagulata* [Say]) in the natural environment infected with *Xf* (i.e. positive for presence of *Xf*) and determining the proportion of those that are infectious (i.e. positive for transmission of *Xf*) (Anderson 1981), greater understanding of the relationship between GWSS densities and *Xf* incidence in vineyards or other plant stands will be obtained. Measurement of GWSS infectivity and infectiousness may prove invaluable in addressing the issue of whether or not there is an upper threshold of GWSS numbers that can be tolerated in a given region.

Information already available indicates that GWSS is relatively inefficient as a vector of *Xf* in a laboratory setting (Almeida and Purcell 2003). However, large numbers of highly mobile vectors such as GWSS can easily make up the difference lost to poor transmission efficiency, especially if a large proportion in the natural environment is infectious with *Xf*. Regional control efforts made over the past few years in areas such as Temecula and the General Beale Road study area in Kern County have proven very effective at reducing local GWSS populations. However, the question of how many of the remaining GWSS in these regions are infectious is still unanswered. Until some measurement is completed of the proportion of GWSS populations that are infected, and more importantly infectious, our understanding of the relative risks posed by variable densities of GWSS throughout California will be limited. More importantly, policy decisions that process information on relative risks posed by GWSS infestations in particular regions will be compromised without data that describes what proportion of a GWSS population is actually causing new infections in a vineyard or in the urban landscape. Better epidemiological information will contribute to improved basic knowledge and understanding and to more sound policy.

The California grape industry remains at the greatest risk of *Xf* movement and transmission by reason of large acreages spread throughout the state and because of the severity of PD. Primary spread of *Xf* into a vineyard occurs when a cicadellid vector such as GWSS acquires the bacterium from a host outside and subsequently transmits to a grapevine within the vineyard. An infected grapevine can then serve (after an unknown latent period) as a source of secondary spread from infected to susceptible grapevines. Because so little is known about the movement of GWSS in the field and when they become infective with *Xf*, it is unknown whether most grapevine infections occur as a result of primary or secondary spread of *Xf*. What is certain, however, is that secondary spread will not occur until a primary infection has occurred, i.e. at least one grapevine has become infected with *Xf*. This is a critical event that poses a high level of risk to the vineyard because of the establishment of a *Xf* source within rather than outside of the vineyard. It is therefore important that all appropriate measures be undertaken to prevent that first critical infection. Towards this goal, it will be most helpful to know the temporal pattern of *Xf* incidence within GWSS populations so that maximum protection can be applied at the most vulnerable times.

The almost complete absence of information regarding the degree of *Xf* incidence in GWSS populations has helped fuel much speculation about the future of the GWSS/PD crisis in California. In reality, there is very little that we understand regarding mechanisms of acquisition and inoculation of *Xf* by GWSS adults, either in the controlled conditions of the laboratory and greenhouse, or in the more challenging setting of their natural habitat. While the laboratory approach can provide essential answers to questions regarding the rate of acquisition and efficiency of transmission, it ultimately reflects the conditions imposed by the researcher. For example, the type and age of the acquisition source plant, the isolate of *Xf* used and period of time that the acquisition source plant has been infected, as well as the source of the experimental GWSS individuals and the conditions under which they are provided access to the *Xf* source plant are all variables controlled by the researcher. A dual approach that balances the findings from the laboratory with monitoring information from the field will improve our understanding of how epidemics of *Xf* occur in vineyards and elsewhere. A compilation of data from many sources has contributed to a good understanding of the distribution of GWSS populations within California and the relative intensities of regional infestations. What is now needed is to determine what proportion of individuals within these populations is infected with *Xf* while also identifying the factors that determine a given level of infectivity. I propose that the approaches and methods to be utilized will address a critical deficiency in our understanding of *Xf* epidemiology, i.e. the proportion of the vector population infected and infectious with the pathogen.

OBJECTIVES

1. Monitor GWSS adults from citrus and other sources year-round to determine the proportion positive for *X. fastidiosa* using ELISA, PCR, and media culturing techniques.
2. Perform transmission experiments on a portion of the field-collected adults using grapevine seedlings to determine the seasonal transmission rate.
3. Quantify the titer of *X. fastidiosa* in GWSS adults that transmitted *X. fastidiosa* to grape seedlings using quantitative ELISA and RT-PCR, and determine the relationship between transmission rate and titer in the vector.

RESULTS

As a new project that began July 2004, progress is being made on gathering the materials for carrying out transmission experiments and detection and quantification of *Xf* in field-collected GWSS. A propagation chamber has been assembled that will enable production of experimental grapevines having homogeneous genotypes to be used in the transmission studies. Lateral branch shoots consisting of 4-5 leaves are being cut from certified disease-free parental grapevines (var. Chardonnay) and placed in propagation media until roots are generated. These are transplanted to 4" pots and allowed a minimum of 3-4 weeks to establish before being used in transmission experiments. Ventilated corsage cages will enclose each grapevine plant and provide full access to the entire plants by GWSS adults. A single adult per plant will be confined 3 days for inoculation access followed by recovery and freezing (-80°C) for PCR and ELISA analysis, or for immediate plating to PD 3 media preceded by surface sterilization. An essential component of each of these approaches will be the availability of clean GWSS that are presently being reared. Experimental grapevines will be held a minimum of 2 months to allow for symptom development and then scored. Xylem fluid will be collected from each plant for ELISA/PCR analysis as an independent evaluation to compare with the visual assessments. Experimental and analytical results will be collated to determine which analytical procedure provides the closest agreement with transmission test results.

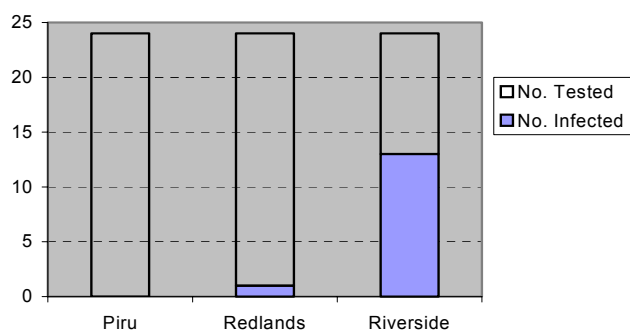


Figure 1. Number of infected GWSS adults from 3 locations collected early October 2004.

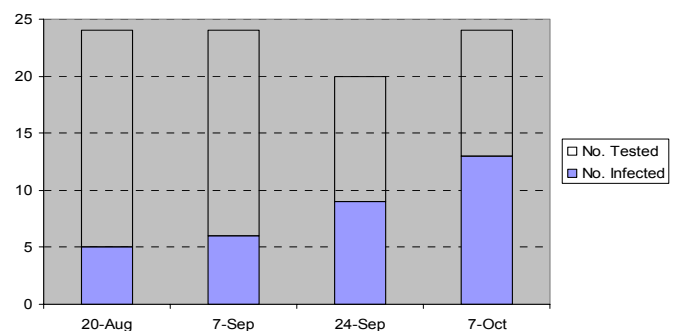


Figure 2. Number of infected GWSS adults out of the number tested for 4 collections from Riverside.

Field collections of GWSS adults that commenced in August 2004 have so far been made in Piru, Redlands, and Riverside. A sub-sample of 24 adults collected from each of these locations in early October 2004 was processed for ELISA detection of *Xf*. More than 50% of the Riverside adults were positive for *Xf* (= absorbance₄₉₀ values > A₄₉₀ mean + 4 standard deviations for the GWSS clean control insects) compared to 4% for Redlands and 0 for Piru insects (Figure 1). A progressive increase in the number of *Xf*-positive insects (Figure 2) occurred between 20 August 2004 (5/24) and 7 October (13/24) in accordance with trends observed from previous years (Naranjo et al. 2003). The distribution of positive A₄₉₀ readings was quite wide,

but with most positives falling in the 0.2—0.6 range (Figure 3). However, a few individuals proved to be highly positive for *Xf* with A_{490} readings >1.0, and in one case >2.4 (Figure 3).

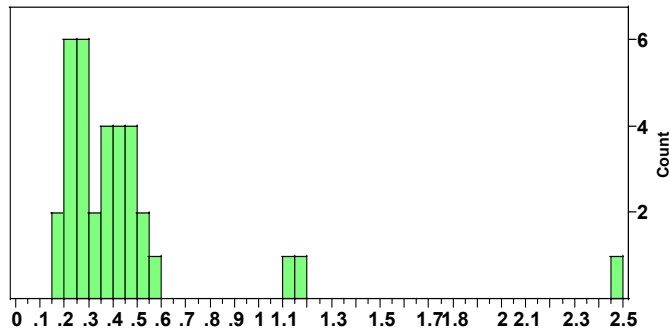


Figure 3. Histogram of Absorbance₄₉₀ readings of GWSS adults collected in Riverside between August and October 2004.

CONCLUSION

The data generated thus far is interesting from the standpoint of the large differences in the number of infected GWSS adults in Riverside compared to Redlands or Piru. As the new summer generation of adults ages, one would expect to find increasing proportions positive for *Xf* as they experience a greater diversity of host plants. This appears to be the case in the Riverside insects, but not for the insects from the other 2 locations. Ongoing collections will help to determine if the location difference is real.

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FUNDING AGENCIES

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QUANTIFYING LANDSCAPE-SCALE MOVEMENT PATTERNS OF GLASSY-WINGED SHARPSHOOTER AND ITS NATURAL ENEMIES USING A NOVEL MARK-CAPTURE TECHNIQUE

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ABSTRACT

Field cage studies were conducted to compare retention times between two inexpensive proteins, non fat dry milk (NFDM) and chicken egg whites, on glassy-wing sharpshooter (GWSS), *Homalodisca coagulata* and *Hippodamia convergens*. Each marker was applied to the insects by either directly spraying the insects with a conventional spraying device or by exposing the insects to pre-marked leaf tissue. Subsequently, the recaptured insects were analyzed by either an anti-NFDM or an anti-egg white enzyme-linked immunosorbent assay (ELISA) to detect the presence of each respective marker. Data indicate that both protein markers were retained well on both insect species, regardless of the application method. Generally, the topical marking procedure yielded higher ELISA values than the insects marked by contact exposure; however, both methods were sufficient for marking almost 100% of each population for > 2 weeks.

INTRODUCTION

Glassy-wing sharpshooter (GWSS), *Homalodisca coagulata* (Say) feeds on a variety of plants, and in the process transmits the bacterium, *Xylella fastidiosa*, which is the causal agent of Pierce's disease (PD) (Varela 2001). The spread of PD by GWSS now threatens the grape and ornamental industries of California. Due to the polyphagous feeding habit and high dispersal capability of GWSS, control of this pest will require an areawide management approach. Such an approach requires extensive knowledge of the host plant preferences and dispersal characteristics of GWSS and its natural enemies. Unfortunately, very little is known about the dispersal characteristics of GWSS (Blua & Morgan 2003, Blackmer *et al.* 2004) and its associated natural enemy complex. This is due, in part, to the lack of an effective technique for studying insect dispersal at the landscape level.

The first phase of our research plan consists of optimizing a mark-capture procedure for GWSS and its natural enemies that will facilitate future studies of intercrop dispersal. Historically, most studies of insect dispersal have relied on the mark-release-recapture (MRR) technique (Hagler & Jackson, 2001). Typically, mass-reared insects or insects collected *en masse* from the field are marked in the confines of the laboratory and then released at a specific site(s) in the field (i.e., at a central point). The insects are then recaptured using various spatial and temporal sampling schemes to quantify their movement. Unfortunately MRR studies use a relatively small portion of the population and recapture even a smaller proportion of the population (i.e., usually < 1.0%), thus making extrapolations about dispersal to the population level less reliable. The information gained from dispersal experiments could be significantly improved if a large proportion of the insect fauna (e.g., the simultaneous marking of GWSS and its natural enemies) could be marked directly in the field (e.g., mark-capture type experiments) and if several distinctive markers were available for studying intercrop movement of insects.

The development of a protein marking technique (Hagler 1997ab, Hagler & Jackson 1998, Blackmer *et al.*, 2004) solved many of the problems associated with other marking techniques for MRR studies. The procedure is simple, sensitive, safe, rapid, inexpensive (for MRR type studies), invisible, and stable (Hagler & Jackson 1998). Moreover, several distinct proteins are available which facilitate the simultaneous marking of different cohorts of individuals (Hagler 1997a, Hagler & Naranjo 2004). We demonstrated that parasitoids (*Eretmocerus* spp. and *Encarsia formosa*) can be easily marked internally with vertebrate immunoglobulin (IgG) proteins by incorporating the various proteins into a honey diet or marked externally (*Trichogramma* sp.) with a fogging device (Hagler 1997b, Hagler *et al.* 2002). However, the major limitation of this technique is that the IgG proteins are too costly for mark-capture type studies. Recently, we discovered two inexpensive proteins that have potential as markers for mark-capture studies. The proteins are casein (from non-fat dry milk) and chicken egg whites (Egg Beaters™ or All Whites™). In collaboration with Vincent Jones we have developed anti-casein and anti-egg white enzyme-linked immunosorbent assays (ELISA) to each of these proteins. In turn, these ELISAs can be used to detect the presence of each protein on protein-marked insects. In this report, we investigated the feasibility of marking GWSS and *Hippodamia convergens* using two different application procedures. The first method for marking the insects consisted of spraying the markers on the insects in the field using a conventional hand sprayer (e.g., direct contact exposure). The second method for marking the insects consisted of exposing the insects to plant tissue that had previously been sprayed with each protein (e.g., residual contact exposure).

OBJECTIVES

The overall objectives of our research are to:

1. Quantify GWSS and natural enemy dispersal patterns in a complex landscape and
2. Determine which factors influence their dispersal. To accomplish these objectives we must first develop a mark-capture protein marking technique and quantify the protein marking retention intervals for the targeted insects. Field application of better mark-capture techniques will enhance our understanding of the area-wide dispersal patterns of GWSS and its natural enemies.

RESULTS

Direct Contact Marking Method

Dozens of nylon-meshed sleeve cages (66 X 70-cm, 18-cm dia.) were placed on randomly selected citrus branches located at the Agricultural Operations Research Station in Riverside, CA. Adult GWSS and *H. convergens* were then introduced into each cage and sprayed with a 5.0% solution of non-fat dry milk (NFDM) or chicken egg whites (All Whites™). A single cage from each marking treatment was randomly selected on 12 different sampling dates for up to 35 days after marking. All of the surviving GWSS and *H. convergens* in the randomly selected cages were assayed by an anti-NFDM or an anti-egg white ELISA to detect the presence of each respective protein mark.

Residual Contact Marking Method

Randomly selected citrus branches located at the Agricultural Operations Research Station in Riverside, CA were sprayed with a 5.0% solution of NFDM or chicken egg whites. The branches were allowed to dry for several hours, and then nylon-meshed sleeve cages were placed on the branches. Adult GWSS and *H. convergens* were then introduced into each cage. The sampling scheme was the same as the one described above. All of the surviving GWSSs and *H. convergens* in the randomly selected cages were assayed by an anti-NFDM or an anti-egg white ELISA to detect for the presence of each respective protein marker.

The ELISA results for the protein marked GWSS are given in Table 1. Data indicate that both marking procedures, regardless of the type of protein marker used, were retained well on GWSS. As expected, the topical marking procedure yielded higher ELISA values and had longer retention than the residual contact marking method. Generally, the markers were retained on 100% of the GWSS for ≈ 2 and 3 weeks by the residual and topical marking procedures, respectively. The ELISA results for the protein-marked *H. convergens* are given in Table 2. *H. convergens* ELISA reactions were very similar to the reactions yielded by GWSS.

CONCLUSIONS

In the first phase of our research described here, we showed that protein markers can be retained on insects several weeks after marking in the field. This marking technique provides the necessary tool to distinguish GWSS and its natural enemies so that studies of dispersal, migration, longevity, and density can be conducted. Additionally, different protein markers can be used to identify insects released at different times, in different areas, or in different crops. Next, we will use this technique to investigate the landscape-level movement of GWSS (nymphs and adults) and its natural enemies. We propose to use the mark-capture system to simultaneously quantify the intercrop dispersal of GWSS and its natural enemies. Specifically, we will spray large areas (e.g., field plots, whole trees, bushes, etc.) with inexpensive proteins using conventional spray equipment. In turn, insects that are hit by the protein solutions or that eat or walk on plant material containing protein residues will obtain enough protein to be detected by protein-specific ELISAs. Because the two marking ELISAs (chicken egg whites and NFDM) do not cross-react, we can apply the materials to two different host plants in close proximity to one another. Then, insects can be collected using temporal and spatial sampling schemes and analyzed for the presence of each respective protein marker to determine not only the insect's point of origin but the timing and extent to which portions of the population move among different plant species.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program and the USDA Agricultural Research Service.

Table 1. The mean (\pm SD) ELISA readings and the percentages of protein-marked GWSS scoring positive for the presence of chicken egg white or non fat dry milk for up to 35 days after marking. GWSS were scored positive for the presence of each marker if the ELISA value exceeded the mean negative control value by 3 standard deviations.

Application Method	Days After Marking	Egg White Marker			Non Fat Dry Milk Marker		
		Number Assayed	Mean ELISA Reading	Percent Positive	Number Assayed	Mean ELISA Reading	Percent Positive
Residual Contact	1	31	0.49 (0.3)	100.0	8	0.38 (0.2)	100.0
	3	7	0.46 (0.4)	100.0	10	0.38 (0.2)	100.0
	5	19	0.94 (0.4)	100.0	4	0.43 (0.1)	100.0
	8	15	0.71 (0.3)	100.0	5	0.20 (0.1)	100.0
	12	26	0.57 (0.4)	88.5	36	0.36 (0.2)	100.0
	13	7	0.52 (0.3)	100.0	5	0.28 (0.3)	100.0
	15	26	0.31 (0.2)	100.0	6	0.27 (0.3)	83.3
	17	13	0.40 (0.2)	100.0	15	0.11 (0.1)	66.7
	19	13	0.17 (0.2)	76.9	5	0.11 (0.1)	40.0
	21	3	0.10 (0.1)	66.7	6	0.08 (0)	66.7
	34	0	---	---	3	0.06 (0)	33.3
	35	13	0.12 (0.1)	46.2	1	0.15 (NA)	100.0
	Negative Controls	25	0.05 (0.01)	0	20	0.04 (0.01)	0
Topical Contact	1	22	1.62 (0.1)	100.0	16	0.43 (0.1)	100.0
	3	12	1.26 (0.6)	100.0	20	0.40 (0.1)	100.0
	5	8	1.13 (0.5)	100.0	1	0.46 (NA)	100.0
	8	13	1.26 (0.4)	100.0	2	0.64 (0.1)	100.0
	12	16	1.23 (0.5)	100.0	8	0.45 (0.2)	100.0
	13	3	0.66 (0.2)	100.0	3	0.41 (0.2)	100.0
	15	3	0.30 (0.1)	100.0	0	---	---
	17	22	0.46 (0.3)	100.0	6	0.38 (0.3)	66.7
	19	7	0.34 (0.3)	100.0	2	0.40 (0.1)	100.0
	21	1	0.07 (NA)	100.0	1	0.04 (NA)	0.0
	34	7	0.16 (0.1)	57.1	10	0.19 (0.2)	80.0
	35	4	0.16 (0.2)	50.0	1	0.49 (0.3)	100.0
	Negative Controls	20	0.05 (0.01)	0	20	0.04 (0.01)	0

Table 2. The mean (\pm SD) ELISA readings and the percentages of *Hippodamia convergens* scoring positive for the presence of chicken egg white or non fat dry milk for up to 35 days after marking. *H. convergens* were scored positive for the presence of each marker if the ELISA value exceeded the mean negative control value by 3 standard deviations.

Application Method	Days After Marking	Egg White Marker			Non Fat Dry Milk Marker ^{1/}		
		Number Assayed	Mean ELISA Reading	Percent Positive	Mean ELISA Reading	Number Positive	Percent Positive
Residual Contact	1	19	0.83 (0.3)	100.0			
	3	19	0.63 (0.2)	100.0			
	5	18	0.29 (0.1)	100.0			
	8	15	0.31 (0.2)	100.0			
	12	12	0.37 (0.3)	75.0			
	13	12	0.49 (0.2)	100.0			
	15	15	0.25 (0.2)	86.7			
	17	5	0.37 (0.2)	100.0			
	19	0	---	---			
	21	3	0.23 (0.2)	66.7			
	34	0	---	---			
	35	18	0.23 (0.3)	94.4			
	Negative Controls	63	0.04 (0.01)	0			
Topical	1	15	1.25 (0.2)	100.0	0.33 (0.1)	17	100.0
	3	26	0.96 (0.3)	100.0	0.34 (0.2)	27	100.0
	5	26	0.62 (0.3)	100.0	0.21 (0.1)	12	100.0
	8	18	0.75 (0.3)	100.0	0.25 (0.3)	2	100.0
	12	33	0.55 (0.3)	100.0	0.17 (0.1)	48	100.0
	13	17	0.23 (0.2)	100.0	0.26 (0.2)	17	100.0
	15	4	0.21 (0.3)	75.0	0.21 (0.2)	8	100.0
	17	20	0.33 (0.2)	100.0	0.25 (0.2)	2	100.0
	19	23	0.24 (0.2)	100.0	0.05 (0.1)	1	33.3
	21	4	0.35 (0.1)	100.0	0.20 (0.2)	20	90.9
	34	23	0.25 (0.1)	100.0	0.11 (0.1)	7	58.3
	35	8	0.27 (0.2)	100.0	---	---	---
	Negative Controls	39	0.04 (0.01)	0	30	0.04	0.01

^{1/}The retention of nonfat milk by contact application was not investigated for *H. convergens*.

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EPIDEMIOLOGICAL ASSESSMENTS OF PIERCE'S DISEASE, AND MONITORING AND CONTROL MEASURES FOR PIERCE'S DISEASE IN KERN COUNTY

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Reporting period: The results reported here are from work conducted from July 2004 to October 2004.

ABSTRACT

Vineyards in the 7 grape production areas of Kern County's area wide management project were surveyed for PD again in 2004. Incidence of PD in the highly affected areas (General Beale and North) peaked in 2002, and declined dramatically in both 2003 and 2004. Treatments to reduce GWSS and to identify and remove PD infected vines each year were associated with these dramatic reductions. Survey and epidemiological data is being processed at CAMFER, a GIS-based research institute at U.C. Berkeley. More than 98% of the vines infected with *Xylella fastidiosa* in the recent epidemic in the General Beale area of Kern County were of the two most susceptible varieties: 6 Red Globe and 2 Crimson vineyards. Thirty-two other nearby or contiguous vineyards of four less susceptible varieties were almost unaffected. A hypothetical mechanism for this varietal difference is proposed.

INTRODUCTION

These two projects have complimentary objectives and methods, and were thus pursued and are being reported here cooperatively. This combination of people and resources has resulted in synergistic efficiency and maximum utilization of resources.

The cooperative area-wide pest management project for the control of GWSS has defined 7 distinct grape growing areas in Kern County. The PD epidemic that peaked in 2002 only affected two of these, the General Beale and the adjacent Northern area. These were also the only areas where the populations of GWSS exploded in 2000 and 2001 to extremely high populations not seen elsewhere in the county. Insect control measures begun in winter 2001-2002 brought the GWSS populations down dramatically. During this time the population dynamics and control methods for controlling GWSS were studied extensively with effective results. However our understanding of how to control the disease (goal of project 1) and the epidemiology of PD when the causal bacterium is transmitted by GWSS (goal of project 2) had been based on limited actual field data. These two projects began in 2002 as 5 year projects to obtain extensive data about the incidence and control of the disease. This disease information would compliment the insect information to enable understanding of the dynamics of the epidemic and methods to control other potential outbreaks. A total of 216 vineyards with 4060 acres and 2,015,698 vines were surveyed, about 4.6% of the vineyard acres in Kern County.

There have been two recent major California epidemics of PD that have been vectored by GWSS: General Beale in Kern County and Temecula in Riverside County. However data about each of these was not obtained until the epidemic was well underway or had already peaked. Because the other five viticulture areas of Kern County did not yet have such high numbers of GWSS, it was thought that disease and insect data from those would provide baseline information in the event that another epidemic such as the General Beale and Northern outbreak might occur, and such an epidemic could be studied from the beginning. Among the other 5 viticulture areas, 4 (Central, South A, South B, and West) have had low numbers of GWSS present since sometime before 2000, and GWSS was discovered in the 5th (Hwy 65-Delano) after 2000. Thus this extensive project to monitor the PD disease incidence in these areas was intended to provide both an understanding of the effect of low populations of GWSS on the incidence of PD, as well as a complete epidemic profile over time if another one should occur in this county.

OBJECTIVES

Project 1: Epidemiological assessments of Pierce's Disease. (BLH)

1. Evaluate the importance of epidemiological factors such as GWSS population size, vine age, cultivar susceptibility, control practices, and GWSS control treatments in vineyards and nearby GWSS hosts or habitat.
2. Make all the epidemiological data obtained available in a commonly acceptable GIS format for analysis by other qualified researchers and epidemiologists.

Project 2: Monitoring and Control Measures For Pierce's Disease In Kern County. (JH)

1. Determine changes in the incidence of PD over time in seven distinct grape-growing areas in Kern County.
2. Develop PD monitoring and management techniques and strategies for use by growers to reduce risk and damage. Update and provide educational materials to assist vineyard managers, pest control advisors, other researchers and government agencies involved in advising growers in the area-wide pest management of the GWSS project.

RESULTS AND CONCLUSIONS

Vineyards were monitored by visually inspecting each vine for PD symptoms, and by collecting and testing (by ELISA) samples from symptomatic vines (2). Thus far in October 2004 all but 2 of the General Beale vineyards have been completed, but much of the other areas of Kern County are still in progress. The results thus far in the General Beale area indicate that the dramatic decrease in the number of infected vines is continuing. From 2002 to 2003 the number of infected vines decreased by 85%, and from 2003 to 2004 the decrease was an additional 68%. Following the survey of these vineyards in 2001 and 2002 the vines found to have confirmed *Xf* infections were removed. The continued decline of *Xf* infection in this area demonstrates that effective PD control can be obtained with a combination of GWSS control, monitoring for infected vines, and removal of infected vines. These projects have demonstrated that vineyard disease monitoring and vine removal is cost effective.

Throughout the county as part of this project vines found to be infected with *Xf* were removed at the end of that season. As a result the surveys in 2003 and 2004 are identifying vines that are newly infected. The rate of infection in all areas of Kern county outside the General Beale and Northern areas is very low, an overall rate throughout the county of less than one new infection per 10,000 vines. By contrast in the General Beale area some of the vineyards developed very high levels of disease within a 2 to 3 year period, peaking in 2002. Several vineyards were entirely lost.

Before the arrival of GWSS, primary spread of *Xf* from sources outside the vineyard accounted for most or all of the PD in California. The rates of new infections in Kern county may be the result of both primary spread and secondary spread, that is vine to vine spread. The low rates of new infections outside the epidemic area is consistent with primary spread, but the rapid rates of infection in many vineyards within the General Beale area is consistent with secondary, vine to vine spread. Perhaps the most startling epidemiological discovery of this project so far was that in 2002, 99% of the PD infected vines in the General Beale area were in Redglobe and Crimson vineyards, the 2 most susceptible of the 6 varieties surveyed. The following year, 2003, these same vineyards accounted for 97% of the diseased vines. These two varieties comprised only 18% of the acreage surveyed in the General Beale area. There were dramatic instances where Redglobe and Flame Seedless were growing in adjacent vineyards, and the susceptible Redglobe vineyards were heavily impacted or totally lost, whereas the more tolerant Flame Seedless vines growing just a few feet away were almost unaffected. The rate of infection in vineyards in General Beale of varieties other than Red Globe and Crimson in any of the three years was less than 14 infected vines out of 337,693 vines surveyed. In the worst epidemic area in Kern County the infection rate in varieties other than Redglobe and Crimson was essentially negligible. The Crimson loss in the General Beale area involved only one vineyard, and these vines were less than three years old. Younger vines are more susceptible to PD than older vines, and it is possible that the losses in the Crimson vineyard were primarily related to their more vulnerable age, rather than a varietal susceptibility. Older Crimson vines may not have been so heavily impacted.

We have developed a new hypothesis that would explain what might be causing this varietal difference. It is based on the timing of when in the season GWSS can acquire *Xf*, when in the season GWSS transmits *Xf* to new vines, and the phenomenon of over-winter curing of *Xf* infections. Over-winter curing of PD has been demonstrated to occur in many areas of California, including the San Joaquin Valley. Populations of *Xf* in grapevines are reduced during the winter dormant season. It has been experimentally demonstrated that if a vine is infected early in the season, the bacterium has enough time left in the growing season to multiply to high enough population levels and spread into areas of the vine where some of the bacterial cells find a refuge and can survive the winter dormancy. The vine then becomes chronically infected and usually eventually dies. Conversely, if a vine becomes infected later in the season, all the bacteria in the vine die over the winter, and the vine is free of disease the following year (1). Also pruning may play some role in over-winter curing. Vines that are inoculated late in the season when there is insufficient time for bacteria to move beyond the inoculated cane would, of course, lose the infection when that cane is pruned. However the bacteria in an un-pruned cane may die over-winter anyway. Our new hypothesis is predicated on the finding that *Xf* multiplies and spreads faster within a susceptible plant than it does in a more tolerant plant (3). It would reasonably follow that the bacterium would also multiply and spread more rapidly in the more susceptible grapevine varieties of Redglobe or Crimson than it would in the more tolerant varieties such as Flame Seedless or Thompson. The first part of our hypothesis is about when in the season a grapevine must become inoculated in order for the bacterium to survive the first winter dormancy in the plant thereby progressing to chronic Pierce's disease. We hypothesize that the tolerant varieties have to become infected with *Xf* earlier in the season than susceptible varieties in order for the bacterium to have enough time left in the growing season to multiply and spread sufficiently in the vine to be able to survive the winter dormancy period. In general it has been demonstrated that vines must be inoculated before some critical time in the season if the bacterium is to survive the winter (1). However the existence of differences among varieties regarding that critical necessary time of inoculation has not yet been experimentally demonstrated.

The second part of our hypothesis is about when in the growing season the bacterial cells, having over-wintered in a previously infected plant, multiply and spread from their winter refuge into the new growth and achieve population numbers great enough to be efficiently acquired by an insect vector, in this case GWSS. This growth and movement of the bacterium following winter dormancy has to happen before vine to vine spread can begin to occur. It is not possible to detect *Xf* in the new growth of an infected plant until sometime about mid-season, and it has been demonstrated that the bacterium must

multiply to relatively high (easily detectable population sizes) before acquisition becomes efficient (4). Because it multiplies and spreads faster, we hypothesize that bacteria become available for acquisition in an infected grapevine of a susceptible variety earlier in the season than in a vine of a tolerant variety.

Putting these two parts of the hypothesis together can explain why the varietal differences in disease rate were observed. In the most susceptible varieties inoculations occurring later in the growing season can result in infections that survive the winter to become chronic. Because of the faster bacterial multiplication and spread there is still enough time in the growing season to reach a threshold for survival. At the same time, the bacteria multiply in previously infected vines fast enough to become available for acquisition by GWSS earlier in the season. The timing of these two processes results in an overlap, that is a window of opportunity when GWSS can acquire *Xf* from an infected vine, transmit the acquired bacteria to a new vine, and the new infection has enough time to progress to chronic infection and disease. That window of time would close during the season, but vine to vine transmissions would still be occurring. However those later season transmissions, after the window of opportunity has ended, would be cured over the winter. So vine to vine transmission occurring within the window would become chronic, and vine to vine transmission occurring after the window would be winter-cured.

Conversely in the tolerant varieties infections must occur earlier in the season in order to have enough time, at the slower rate of multiplication and spread, to progress to chronic disease. At the same time bacteria from previously infected vines also multiply and spread slowly and do not become available for vector acquisition until later in the season. The result is that there is no overlap, no window of opportunity where GWSS can acquire *Xf* from an infected vine, transmit to a new vine, and have the newly infected vine progress to chronic disease. In this case all of the vine to vine transmissions occur too late in the season, and the result is that all the vine to vine infections are cured over the winter.

One question is why do epidemics that are vectored by GWSS result in vine to vine disease spread in susceptible varieties whereas no vine to vine disease spread seems to occur when the traditional native California sharpshooter vector species are transmitting the bacterium? The answer may be related to the feeding and inoculation locations of GWSS vs. other vectors. The GWSS will feed (and therefore inoculate vines) at the base of the canes, but the native vectors all feed almost exclusively at the tip of the cane. Inoculations at the tip of the cane probably require more time to move to an over-wintering refuge, so an early season inoculation is necessary for the infection to survive the winter and become chronic disease. Thus the window for vine to vine transmission leading to chronic disease would not exist. In this case only the early season primary spread from sources outside the vineyard would result in chronic disease, and because vine to vine transmission cannot begin until mid-season, these infections would be winter-cured.

If this hypothesis is correct, there are a number of possible consequences and conclusions that could improve PD management and control in areas where GWSS is present.

- The risk to growers of tolerant varieties is far less than has been previously assumed.
- There is a critical window of time somewhere in mid-season when susceptible vines need to be protected from vine to vine spread of PD. Chemical vineyard treatments early and late in the season, that is before and after this window, may be less effective than has previously been assumed.
- Economically important rates of secondary spread of PD may only happen in susceptible varieties and when large populations of GWSS are involved. Low but persistent populations of GWSS in Kern County do not appear to have resulted in appreciable losses from vine to vine spread.
- Better targeted and timed chemical treatments could result in lower costs and be more compatible with other IPM programs.
- Late season vineyard surveys and rouging of infected vines is an important and cost effective management tool.
- The GWSS monitoring programs could be tailored to critical parts of the season, thereby possibly reducing the overall cost of these programs.
- The GWSS population treatment thresholds could be based on better epidemiological information, again possibly reducing overall PD management costs.

Because of the beneficial implications for PD management, it is important to experimentally test this hypothesis. We will be proposing to conduct experiments over the next two years to test the components of this hypothesis. The best experimental protocol would involve experiments conducted in two adjacent working vineyards, one tolerant and one susceptible variety. Ideally the experimental site would be in southern San Joaquin valley with climatological conditions representative of the viticulture areas of Kern or Tulare counties. One experiment would involve inoculations of both varieties vines at intervals throughout the growing season to establish the probability curves for the over-winter survival of *Xf* as a function of time of inoculation. The hypothesis predicts that the probability curves would be significantly different. Another experiment, for year two, would involve acquisition of *Xf* by GWSS at intervals throughout the season from vines of both varieties that were inoculated the previous year. This would establish the probability curves for the acquisition of *Xf* by GWSS as a function of time. The hypothesis predicts that these probability curves would also be significantly different. Other components of the experiments would look for differences between the varieties in the rate of multiplication and spread of *Xf* in the vines. Again the hypothesis would predict differences. It is critically important to everyone involved that these experiments do not create any new local PD problems or outbreaks. We have considered extensive safeguards in the design of these

experiments. We intend for the risk to be very small, and the knowledge gained to be of great benefit in the practical control of PD in the southern San Joaquin and elsewhere in California. We would be happy to work collaboratively with other researchers and cooperators on various aspects of this research.

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FUNDING AGENCIES

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SPATIAL DATABASE CREATION AND MAINTENANCE FOR PIERCE'S DISEASE AND GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA

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Reporting Period: The results reported here are from work conducted from July 1, 2004 to October 1, 2004.

INTRODUCTION

Whether tracking invasive species, assessing water quality, or monitoring the spread of disease, comprehensive data collection is a key component of scientific inquiry and sustainable natural resource management. Geographic Information Systems (GIS) allow us to unite in one structure spatially referenced data with other information, affording new insights in relationships between variables at multiple scales (original proposal contains full references), as well as assisting in collaborative efforts at natural resource management and multi-disciplinary problem solving. Such is the case with Pierce's Disease, where disparate datasets on PD location and GWSS trap data could, if available in a Geographical Information System (GIS) format with other spatially referenced data "layers" such as crops, hydrography, climate, and roads, aid in management of the disease, as well as in epidemiological research.

Several agencies and individuals have recognized the need for such a geospatial database for PD research and management. Indeed, the University of California Agriculture and Natural Resources "Report of the Pierce's Disease Research and Emergency Response Task Force(<http://danr.ucop.edu/news/speeches/executivesummary.html>) lists the following recommendations: Support is needed for a coordinated, statewide monitoring, trapping and reporting program involving governmental agencies, the agriculture and nursery industries and UC. The objective is to locate populations of GWSS and BGSS, track the incidence and distribution of Pierce's disease and carry out emergency response programs to slow the spread of PD and its vectors. CDFA or UC should manage a GIS to store, display, manipulate and overlay information collected by statewide monitoring and tracking programs. This data should be available to decision makers, growers and scientists.

We propose to develop a statewide database for PD and GWSS, maintaining the data with the best QA/QC methods, and full metadata (for data ownership tracking), maintained in a GIS format. We also propose to build a mechanism for researcher access to the database via the web, so that data can be downloaded for research purposes, and uploaded to the collection. We are not linking this effort with any analytical proposal, but aim to create the best possible, accessible database for others to use in research. These two components: (1) GIS database storage and maintenance and (2) Internet accessibility, when combined, are called "webGIS", and although not yet widely used in natural resource management, such systems are a promising option for entering and storing heterogeneous datasets, indexed by location, and making them widely available in a visual, dynamic, and interactive format. We use as our model the Sudden Oak Death monitoring project (please see the website at: <http://kellylab.berkeley.edu/SODmonitoring>) created by the Project Leader M. Kelly and housed at UC Berkeley.

The multi-scale data provide by the database structure described here, and specifically the access to the data, will contribute to finding a solution to PD by allowing researchers to use PD and GWSS data in concert with other spatial data "layers" such as climate, crops, and roads. In this way epidemiological hypotheses about distribution and spread at several scales – from vineyard to county to regional - can be formed. In addition, the data will aid in disease management, as researchers can see the spatial effect of different management options such as vine removal.

We are committed to collaborate with relevant researchers in this pursuit, and understand that there are already existing groups collecting such data. It is not our wish to supercede those efforts, but to lend our expertise to the data collection, storage, and distribution dynamic in support of Pierce's Disease science.

OBJECTIVES

The objectives and priorities for this project are as follows:

1. Create spatially referenced database of PD occurrence from field data;
 2. Create spatially referenced database of GWSS trap data;
 3. Maintain these data with other relevant spatial data for researchers use; and
 4. Develop a web-based tool for researchers to submit data to the database, and for researchers to access existing data.
- Possibly, we will also develop a tool for the public to report presence of GWSS.

RESULTS

Funding for this project arrived at UC Berkeley on October 11, 2004, so we have no specific data analysis to report. I have a Staff Research Associate – Dave Shaari – who will work half time on this project, and I am in the process of locating an

undergraduate to assist. The data storage and web server is currently on order. I plan on presenting the plan for this database with PD investigators at the December conference.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

IMPROVING DETECTION OF PIERCE'S DISEASE INFECTED GRAPEVINES

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ABSTRACT

Monitoring grapevines for Pierce's disease (PD) is an important component of disease management and epidemiology research. Currently, there are no guidelines for how to choose plant tissue from grapevines for detecting diseased vines. This study was initiated to develop criteria to increase the likelihood of detecting grapevines infected with PD. Grapevines naturally infected with PD were identified from vineyards in the Coachella Valley and Temecula, California. Grapevine canes were removed from three vineyards with three different grape varieties: Perlette, Superior Seedless, and Chardonnay. The probability of detecting a PD-positive cane was greater in petioles tested from basal portions of canes. No differences were found between healthy and PD-infected canes in internodal distance, petiole weight, petiole length, or the number of leaves occurring at branches on canes. In preliminary observations, 9.5% of petioles from PD-infected vines were PD-positive, but had asymptomatic leaves and 16.1% of petioles were PD-negative, but had symptomatic leaves. Healthy vines had 16.7% of petioles with symptomatic leaves that were PD-negative. Symptoms were more apparent on leaves from basal cane portions and asymptomatic PD-infected petioles were more common on distal cane portions. Image analysis to confirm these results is in progress.

INTRODUCTION

A major component of Pierce's disease (PD) research in California has been grapevine sampling to monitor PD incidence in vineyards. Identification of PD-infected vines is important for management and investigating disease epidemiology. University of California guidelines for management suggest removal of chronically infected vines to reduce the possibility of secondary disease spread and increase vineyard productivity by replanting with healthy vines (Varela et al. 2001). Relatively new programs in Kern County (Hashim et al. 2003) and the Coachella Valley (Perring et al. 2003) have been implemented to monitor PD in areas where it had been thought to be uncommon. Most PD monitoring programs have been based on preliminary identification of infected vines based on PD symptoms (Hashim et al. 2003, Perring et al. 2003). Unfortunately, PD symptoms can be similar to other grape diseases and nutrient deficiencies (Varela et al. 2001) and diseased vines may be asymptomatic early in disease progression. To definitively identify infected vines, plant tissue should be tested by a reliable diagnostic method such as culturing, enzyme-linked immunosorbent assay (ELISA), or polymerase chain reaction. Protocols for sampling to detect infected vines in vineyards are needed to reliably detect PD. A first step to preparing such a protocol is determination of the best approach for choosing plant tissue for diagnostic tests.

OBJECTIVES

1. Determine the probability of detecting a PD positive vine based on petiole location on individual grape canes.
2. Compare the morphology of healthy and PD-infected grape canes for potential differences that could aid in identifying infected vines.
3. Evaluate the effectiveness of using PD foliar symptoms for choosing plant tissue for diagnostic tests.

RESULTS

Naturally-infected grapevines with PD were identified from two vineyards in the Coachella Valley and one vineyard in Temecula. Varieties at the three respective locations were Perlette (3 vines), Superior Seedless (6 vines), and Chardonnay (5 vines). Three canes from each vine were removed. Each leaf from the canes was photographed, and intact individual petioles were weighed and tested for PD by ELISA. Additionally, in the Coachella Valley three canes were harvested from

two non-infested vines of each variety. On all canes from the Coachella Valley, internodal distance and petiole weight were measured and the number of leaves occurring at cane branches was counted.

Probability of PD Detection Based on Petiole Location

The probability of detecting PD from an individual petiole was greatest in basal portions of the cane (Figure 1). This result follows the suggestion of Hill and Purcell (1995) that the newest growth would not likely contain bacteria because of the incubation time required for spread. Our result is likely most applicable to chronic infections and this has been noted by others (Feil et al. 2003), but not presented by our method of examining infection on a node basis along the length of entire canes.

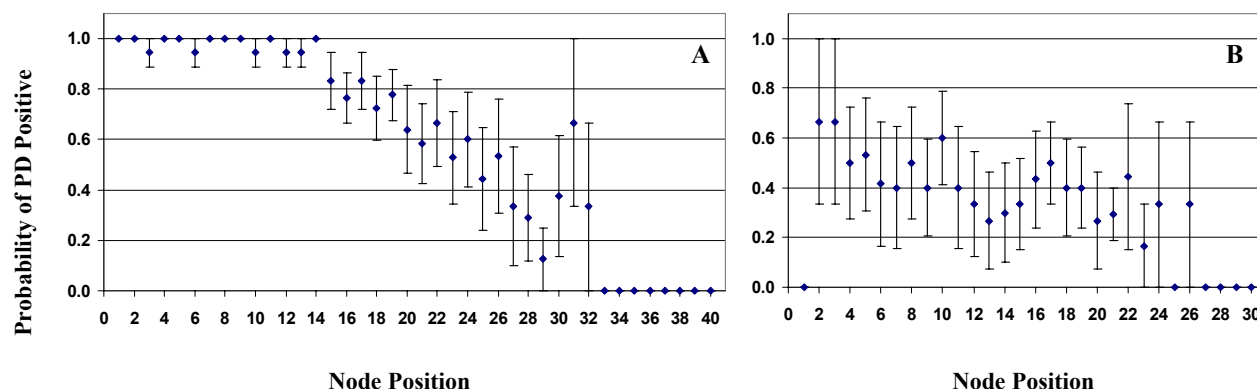


Figure 1. Probability (\pm SE) of positive PD detection at each node position (1 is most basal) for (A) Superior Seedless ($n=6$) vines and (B) Chardonnay vines ($n=5$).

Morphology of Healthy and PD-infected Vines

We did not detect any differences in Perlette ($A=0.57$; $df=4, 11$; $P>0.05$; MANOVA) or Superior Seedless ($A=0.89$; $df=4, 11$; $P>0.05$; MANOVA) varieties in internodal distance, petiole weight, petiole length, or number of leaves branching off of canes between healthy and infected canes. We measured these factors with the intent to identify a morphological feature that could aid in identifying infected vines, but no differences helpful for this purpose were found.

Effectiveness of PD Symptoms for Sampling

We photographed each leaf from each cane to evaluate the reliability of symptoms for use in identifying PD infected vines. We have begun to examine the visual symptoms in relation to PD infection and will use image analysis to quantify foliar symptoms. In preliminary observations, 9.5% of petioles from PD-infected vines were PD-positive and had asymptomatic leaves, and 16.1% of petioles were PD-negative, but had symptomatic leaves. Healthy vines had 16.7% of petioles with symptomatic leaves that were PD-negative. Generally, symptoms were more severe in basal portions of canes and the likelihood of finding an asymptomatic positive petiole was greater on distal portions of canes (Figure 2).

CONCLUSIONS

- Samples taken from basal portions of grapevine canes were more likely to yield an ELISA positive result. We believe this result applies primarily to chronically infected vines.
- We did not discover cane morphological differences between healthy and PD-infected vines that could be useful in detecting PD infected vines.
- We are in the process of evaluating the relationship between PD foliar symptoms and PD infection and have observed that the likelihood of a PD symptomatic leaf being negative for PD was greater than the likelihood of a PD asymptomatic leaf being positive for PD. Also, distal portions of canes were more likely to be asymptomatic when infected with PD.
- Based on the potential for choosing symptomatic leaves that are PD-negative, we suggest taking petiole samples for PD diagnostic tests from basal portions of grape canes to increase the likelihood of detecting PD positive vines.

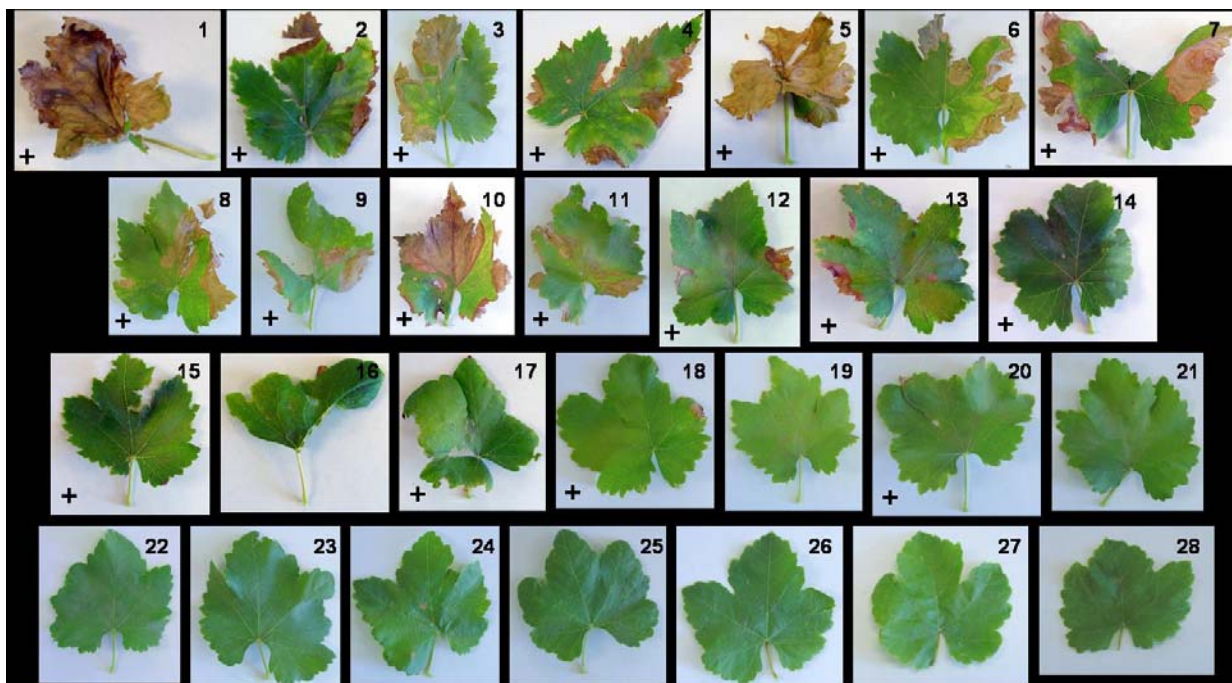


Figure 2. Individual leaves from a single Superior Seedless cane. Number indicates node position with 1 being the most basal node. The plus symbol indicates that the petiole from the leaf tested positive for PD by ELISA.

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TREATMENT THRESHOLDS FOR THE GLASSY-WINGED SHARPSHOOTER BASED ON THE LOCAL EPIDEMIOLOGY OF PIERCE'S DISEASE SPREAD (A STAGE-STRUCTURED EPIDEMIC MODEL)

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ABSTRACT

The conditions for the successful invasion of a vineyard by Pierce's disease (PD) are not well understood. To help integrate what knowledge we do have and indicate areas where research is needed we are developing a more biologically detailed model than has been previously available. Fortunately there is a large ensemble of literature from epidemiology regarding this problem, and in addition, much has been done toward solving the kinds of equations that arise in this work in terms of both mathematics and software. Here we outline very briefly our progress to date, and the ways in which these sorts of models can help us to better manage and understand the PD system. Here we describe a system of delay equations for modeling the dynamics of PD vectored by the glassy-winged sharpshooter (GWSS). We will analyze and study this system to derive threshold conditions for the invasion of a vineyard by PD and GWSS. Thresholds for disease outbreaks are common among epidemiological systems and a large literature exists on this subject. In addition new software (not commercially released yet) has been made available to us for solving these kinds of systems. We will attempt to use our model system to bring this methodology to the PD/GWSS problem and find new ways of controlling this disease.

INTRODUCTION

Last year we presented a model to evaluate how the threshold might change in relation to various biological and ecological factors (Perring et al. 2003). It was designed to determine the number of GWSS required to cause a single PD infection in grape. The primary model parameters were the proportion of GWSS carrying PD, GWSS transmission efficiency of PD, proportion of GWSS that will move from citrus to grape, the number of grapevines that a single GWSS will visit, grape varietal susceptibility, and the probability of an infection event resulting in disease. Our recent work, reported in this progress report, is an extension of the previous efforts and is more biologically detailed, allowing us to address more complicated biological processes affecting the epidemiology of PD in grapes. Over eighty years of research in epidemiology has shown that epidemics tend to be triggered when the generation reproductive factor of the pathogen becomes greater than 1.0 (Kermack and McKendrick 1927, Anderson 1978, Diekmann and Heesterbeek 2000, van den Driessche and Watmough 2002, Wonham et al. 2003). This fortunate result is useful in management since it provides us with a target threshold that will trigger a PD epidemic in grapes. More than just a threshold, this approach will provide a function for the basic generation factor of increase of the pathogen, R_0 , as a parameter function from the model. The pathogen will grow into an epidemic or decline to zero according to whether R_0 is greater or less than 1.0. It is particularly helpful that this threshold indicator is a function of all of the model parameters, since this indicates what parameters the threshold is most sensitive to and therefore how management can be most effectively focused. Some of the things that we intuitively expect to be important are density of GWSS, pathogen titer of the insects, and their dispersal rate and feeding rate.

OBJECTIVES

1. Develop a model to describe the epidemiology of GWSS transmission of PD to provide a framework for organizing data and examining relationships between data from different research projects.
2. Use the model to develop field-specific treatment thresholds to prevent GWSS transmission of PD.

RESULTS AND CONCLUSIONS

Our results consist of a model system of state equations describing the progress of PD in a vineyard vectored by GWSS. Here we develop our basic model as set of four balance equations, two equations for the GWSS and two equations for grapes.

The state variables, process functions and parameters are defined in Table 1. We emphasize that this model is in an early development stage, and undoubtedly will evolve and improve as we develop it further. We used the delay-differential equation (DDE) formalism developed by Murdoch et al. (1987) and Murdoch et al. (2003) for stage structured insects, and to their formulation we will add time dependence (temperature forcing) of the developmental delays (although for simplicity we will not elaborate on this here). The time dependence in the delays can be incorporated according to the mathematical recipes developed by Nisbet and Gurney (1983), Gurney et al. (1983), Gurney and Nisbet (1998) and Nisbet (1998). Methods for setting up the initial history for starting the models are outlined well in Gurney et al. (1983). We will solve our set of equations using a new delay differential equation (DDE) solver, ddesd.m, (with time and system varying delays) developed for The Mathworks (Matlab) by L. F. Shampine (Shampine & Thompson 2001, Shampine 2004). The solver is not yet a part of Matlab itself, but a version is available on the Web at: <http://faculty.smu.edu/lshampin/current.html>.

Our model system.

The state balance equations are written as a set delay-differential equations (DDEs) with functions for recruitment, infection and death rates as:

$$\begin{aligned}
 \text{Susceptible Adults: } \frac{dA_s(t)}{dt} &= R(t - T_J)S_J - X(t) + X(t - T_1)S_1 - D_A(t) \\
 \text{Infectious Adults: } \frac{dA_I(t)}{dt} &= X(t) - X(t - T_1)S_1 - D_A(t) \\
 \text{Susceptible Vines: } \frac{dS(t)}{dt} &= D_V(t) - Y(t) \\
 \text{Infectious Vines: } \frac{dI(t)}{dt} &= Y(t - T_2) - D_V(t)
 \end{aligned} \tag{1.1}$$

We adopted and slightly modified the notation of Murdoch et al. (2003) by using $R(t)$, $X(t)$, $Y(t)$ and $D(t)$ to represent recruitment (R), infection of GWSS (X) infection of vines (Y) and death rate (D) functions for each stage, and we then define each of these for our case. These equations indicated that the rate of change of a stage is simply the input to that stage minus output from that stage. The interpretations for each equation are outlined below.

Susceptible adult equation.

The first equation says that susceptible adults have input from reproduction, one juvenile delay period (T_J) in the past times survival going through the juvenile stage, $R(t - T_J)S_J$. Another input to susceptible adults is (possible) recovery from an infectious adult class with a time delay, $X(t - T_1)S_1$ where T_1 is the time that the disease persists in an infected adult, and S_1 is the survival during the infectious period. Outputs from susceptible adults are infection by feeding on an infectious vine, $X(t)$, and death, $D_A(t)$.

Infectious adult equation.

The second equation says that infectious adults have input from the infection process, $X(t)$, (which was output from the susceptible class) and output to (possible) recovery from infection, $X(t - T_1)S_1$, and death, $D_A(t)$.

Susceptible vine equation.

The third equation says that susceptible vines have input equal to death rate of infectious vines, $D_I(t)$, that is, we assume that dead vines are replaced at the death rate. Output from susceptible vines is infection by infectious sharpshooters, $Y(t)$.

Infectious vine equation.

The last equation says that infectious vines have input from the infection process with a latent period time lag, $Y(t - T_2)$, where T_2 is the latent period of the disease in vines after becoming infected. We assume that all vines survive the latent period. Output from the infectious vine equation is by death of infected vines, $D_I(t)$.

Our model system of equations will allow us to simulate the introduction and progress of PD into a vineyard under different conditions and management strategies. What we would like is to see the disease die-out and not invade the vineyard effectively. What we do not understand at this point is how all of the factors influence this scenario and determine its progress and to which factors spread is most sensitive. By studying the dynamic behavior of this model system we can learn how different management options are likely to affect the disease progress in a vineyard, giving us new ideas and methods about how to best control and prevent disease outbreaks.

Table 1. State variables, process functions and parameters for GWSS-PD Model

Variables	Description	
$A_s(t)$	Susceptible GWSS Adults	
$A_i(t)$	Infectious GWSS Adults	
$S(t)$	Susceptible Vines	
$I(t)$	Infectious Vines	
Process Functions		Process Sub-Models
$R(t - T_j)S_j$	Recruitment into the adult stage	$R(t - T_j)S_j = b(A_s(t - T_j) + A_i(t - T_j))S_j$
$D(t)$	Death rate for a stage	Linear constant death rate, e.g.: $D_A(t) = d_A A(t)$
$X(t)$	Infection rate for GWSS	$X(t) = \alpha I(t)A_s(t)$
$Y(t)$	Infection rate for vines	$Y(t) = \beta S(t)A_i(t)$
S_j	Survival of stage J with constant death rate	$S_j = \exp(-d_j T_j)$
Parameters		
b	Average birth rate	
T_i	Time in the i th stage or process	
d_i	Constant death rate for i th stage	
a	Transmission rate for GWSS	
β	Infection rate for vines	

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DEVELOPMENT OF A FIELD SAMPLING PLAN FOR GLASSY-WINGED SHARPSHOOTER-VECTORED PIERCE'S DISEASE

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ASBTRACT

Determining the location of grapevines infected with Pierce's disease (PD) in vineyards has been a major question for growers and researchers. Field census has been the only reliable way to identify vines infected with PD in the vineyard. Censuses, however, are difficult when PD incidence is high. In these situations, we need a sampling program that accounts for the spatial structure and pattern of PD in the vineyard. To characterize the spatial distribution patterns of PD, census data from Kern County vineyards were analyzed with geostatistics. These analyses showed that dispersion of PD varied with the amount of PD infection, and with vineyard proximity to citrus. Based on these analyses, our goal is to develop a sequential sampling program for detecting PD in vineyards.

INTRODUCTION

A common sampling technique to detect the presence of PD in vineyards is to visually examine vines, remove symptomatic leaves from possible infected vines, and confirm the presence of PD with enzyme-linked immunosorbent assay (ELISA). Locating vines infected with PD in a vineyard is required for current PD management, and the only reliable method for locating PD-infected vines is to examine every vine in the vineyard. Such a census was used for a county-level PD survey and provided a cost-effective method (< \$5 per acre) for identifying infected vines in vineyards when PD infection was very low (Hashim and Hill 2003). As the infection level in a vineyard exceeds 1%, it becomes more difficult to observe and sample every symptomatic vine. It is especially difficult to distinguish PD symptoms when other stress factors, such as drought and salt damage, exist in vineyards. Such difficulties result in high sampling costs because many samples must be taken and confirmed with ELISA. Thus, the development of a cost-effective sampling program appropriate for growers' and researchers' needs and skills is necessary for PD monitoring and management.

By definition, a sampling program employs all available sampling techniques to collect samples that are used to make estimates of population parameters (Pedigo 1994). In our case, we need to estimate the distribution and abundance of PD-infected vines. The sampling techniques consist of the actual equipment and methodologies by which samples are collected (Pedigo 2002). Sampling programs, on the other hand, direct how often and how many samples are to be taken, the spatial pattern to obtain sample units, and the timing of sampling (Pedigo 1994). Sampling programs often include binomial sampling or sequential sampling that makes sampling more cost effective and convenient. However, in PD sampling, such sampling plans cannot be directly adopted because the purpose of PD sampling is not only to estimate the incidence of PD but also to locate individual vines infected with PD. Thus, the sampling program for PD should be spatially oriented to identify the locations of the individual vines infected with PD.

One way to locate infected vines without a census is to use sampling grids that match the spatial structure and patterns of PD distribution. To develop these sampling grids three facts should be known: 1) the spatial structure and patterns of PD distribution, 2) the relationship between PD distribution and the percentage incidence of PD, and 3) the relationship between PD distribution and environmental factors affecting the incidence and spatial distribution of PD. Such knowledge can be obtained with current technology and methods such as the global positioning system (GPS) to locate sampling grids, the geographic information system (GIS) to generate geo-referenced data, and geostatistics analyze spatial data.

OBJECTIVES

The goal of this project is to develop a sequential grid-sampling program for PD that can characterize the spatial distribution and determine the location of PD based on the spatial structures and patterns of PD distribution in the vineyard. The objectives of this project include:

1. Characterization of the spatial distribution of PD in vineyards.
2. Development of a sequential grid-sampling program.
3. Validation and optimization of the sampling program with cost analysis and sensitivity analysis.

RESULTS AND CONCLUSIONS

We have conducted censuses of Kern County vineyards for the past four growing seasons (2001-2004). This report is focused on the 2002 data. Census data were converted into a GIS database and analyzed with geostatistics. Geostatistics is a set of statistical procedures that can characterize distribution (called *semivariogram modeling*) and generate distribution maps (called *kriging*). The semivariograms show the spatial pattern (e.g., no structure, uniform, trend, random, or clumped) and the structure (e.g., the size of aggregation, spatial correlation, and spatial variability) of PD distributions. Kriging was used to generate distribution maps of the probabilities of PD infections throughout the vineyard.

Census result

We made a census of 215 vineyards in 2002. A total of 135 vineyards were infected with PD. Only seven vineyards had more than 0.1% PD infection, and those vineyards were located adjacent to citrus groves indicating that citrus affects the incidence and severity of PD in nearby grapes. This result is consistent with patterns of PD found in Temecula (Perring et al. 2001). However, as in the Temecula study, proximity to citrus did not affect PD distribution in all Kern County vineyards.

Spatial distribution of PD in vineyards

Determining distribution patterns (e.g., no structure, uniform, trend, random, clumped) is the first step for developing sequential grid-sampling plans for fields in which we do not know the location of infected vines. Geostatistical analyses showed that the distribution pattern of PD could be categorized according to the incidence of PD in each vineyard. When the infection was < 0.1%, there was no spatial structure to the location of infected vines. Vineyards that had between 0.1% and 1% infection showed a distribution pattern of a trend from areas of high infection to low infection (Figure 1A). This type of distribution pattern (i.e. trend) also was found in the Coachella Valley in a field that had a similar proportion of infected vines (Figure 2). When the infection was between 1% and 5%, the pattern of disease was random (Figure 1B), and a clumped distribution existed when infection rate was > 5.0% (Figure 1C).

Our work suggests that knowing the percentage of PD infection and the location of vineyards relative to citrus can predict the distribution pattern of PD in the vineyard. Such inferences from the geostatistical analysis can be used to develop a spatially-oriented sampling program with sampling grids. The development of this sequential grid-sampling program provides three fundamental roles in PD research and management. First, it enables growers to locate vines infected with PD in the vineyard when the proportion of infected vines precludes a vineyard census. Second, using with the geospatial and geostatistical methodologies of the sampling program, growers will be able to identify problem areas in their vineyards. Third, the sampling program provides a method for standardizing PD sampling statewide. Progress in these areas, i.e. locating individual vines, identifying problem areas in a vineyard, and standardizing areawide monitoring, not only will help growers make informed decisions in their own vineyards, but will assist researchers trying to understand the epidemiology of GWSS-vectored PD in California.

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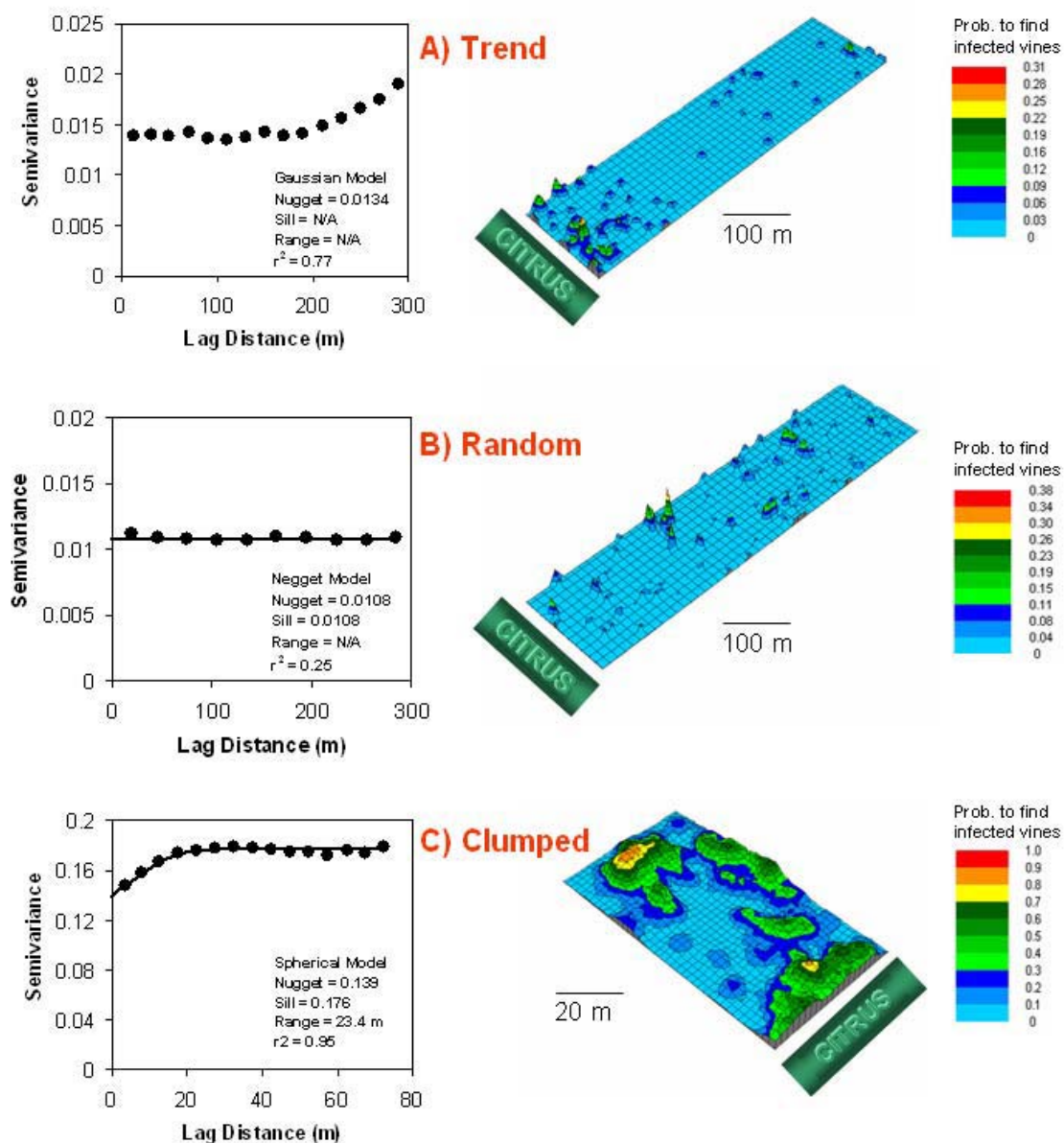


Figure 1. Three main dispersion patterns of PD found in Kern County in 2002. (A) A “trend” spatial pattern from areas of high infection to low infection existed when the infection was between 0.1% and 1.0%. (B) A “random” distribution pattern existed, when the infection was between 1% and 5%. (C) A “clumped” dispersion pattern existed when PD infection was > 5%. When infection was < 0.1% there were no detectable spatial structures.

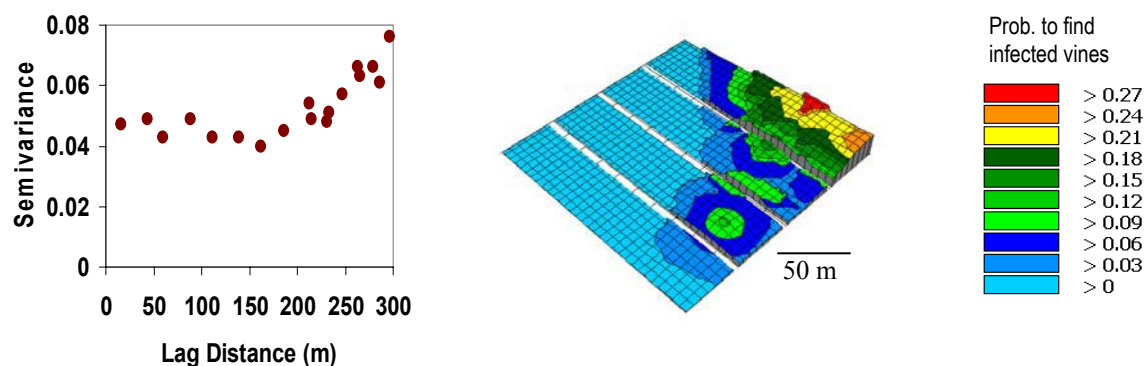


Figure 2. Semivariogram and dispersion map for PD in a Coachella Valley vineyard. The semivariogram indicates a trend dispersion pattern. Within this trend, a random dispersion pattern exists up to a lag distance of 200m. This trend from high to low PD is easily visualized in the dispersion map.

DETECTION OF *XYLELLA FASTIDIOSA* IN INSECT VECTORS IN CALIFORNIA

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ABSTRACT

Recent spread of *Xylella fastidiosa* (*Xf*) to several agricultural commodities and ornamental plants in California has prompted great interest in understanding the comparative interactions between *Xf* and native and recently introduced insect vectors. The generally low titer of *Xf* in insect vectors limits the use of serological techniques, such as ELISA, for qualitative and quantitative analyses of *Xf* associated with different insect vectors. *Xf* detection by molecular techniques, such as PCR, can potentially overcome this limitation. The objective of this study was to compare standard PCR for detection of *Xf* in field-collected insects as well as in greenhouse-reared insect vectors using primers RST31/RST33 with newly developed primers HL5/HL6 in standard PCR and in Real Time PCR using the system HL5/HL6 and a probe labeled with FAM. Two native species the green sharpshooter (*Draeculacephala minerva*) and the red-headed sharpshooter (*Xyphon fulgida*), and the recently introduced glassy-winged sharpshooter (*Homalodisca coagulata*) were included in this study. Field-collected insects were obtained from *Xf*-infected grapevines and almonds in the San Joaquin Valley, California. Greenhouse-reared green and red-headed sharpshooters were also obtained from cultures maintained on a non-host of *Xf* in Parlier, California. Five-10 *Xf* cells per μL of insect head DNA sample were detected with the HL5/HL6 primer pair-FAM system. Also, using this system, the number of *Xf*-cells detected in field-collected and greenhouse reared insect was between 10^2 - $10^3/\mu\text{L}$ sample/reaction. This concentration of *Xf* cells was detected by visualization of the *Xf*-specific amplicon (221 bp) in gels following standard PCR with the HL5/HL6 primers. This level of pathogen in insect heads was below the limit of detection in standard PCR with primers RST31/RST33. Using Real-Time PCR quantification with the system HL5/HL6-FAM, the total amount of *Xf*-cells per insect head was estimated to be between 10^4 - 10^5 . Implications of these results on the epidemiology of the disease are discussed.

EVALUATION OF A NOVEL, FIELD DEPLOYABLE, ELECTROCHEMICAL DETECTION SYSTEM FOR THE DETECTION OF *XYLELLA FASTIDIOSA* WITHIN GRAPEVINE PETIOLES

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ABSTRACT

We have tested a new electro-chemical detection (ECD) system designed by AnzenBio, Inc. for the quick detection of *Xylella fastidiosa* within grapevine petioles. Like standard ELISA this detection method relies on antibodies against the bacterium, but unlike ELISA it detects movement of electrons through the final product conversion, measuring current rather than color change. Using a hand-held meter and pre-coated chips the test can be done in a fraction of the time (1.5 vs. 5 hrs.). Comparison of 18 Cabernet Sauvignon petioles from a vineyard with Pierce's disease (PD) to 18 petioles guaranteed PD free showed the ECD readings per gram of tissue to be higher for PD petioles (31.3 vs. 6.2 microamps). This difference is statistically different using a t-test ($p < 0.0001$). In another trial in South Texas, ECD was used to evaluate the petioles from three different varieties, Blanc du Bois, Black Spanish and Cynthiana, which have been shown to carry differing levels of *Xylella fastidiosa* within this area of high PD pressure. Petioles were also categorized into those from leaves with low, medium and high PD symptoms. Analysis of variance on ECD data from the 9 symptom variety categories with 6 replications showed that ECD could detect distinct significant differences between several of the categories ($p < 0.0001$). Analysis of variance on ELISA data run on the same 54 samples found no significance between categories ($p = .43$). ECD appears to give more sensitive readings over a range of bacterial levels, potentially giving fewer false positives.

